Reaction sequence of blood coagulation

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Reaction Sequence of Blood Coagulation

In 1964 Macfarlane[1] proposed a reaction mechanism for the interaction of blood coagulation factors, known as the enzyme cascade. In this hypothesis the blood coagulation factors as they occur in plasma are considered to be zymogens (except factor I; namely, fibrinogen). Coagulation is initiated by the conversion of one of these zymogens into an enzyme as a result of contact with anything other than intact vascular endothelium. This enzyme then acts on another clotting factor to form another active enzyme. All clotting factors thus interact in a given order until prothrombin (factor II) is converted into thrombin. Thrombin then brings about the actual coagulation by converting fibrinogen into fibrin.

The prothrombin converting activity (prothrombinase activity) was first thought to be exerted by activated factor V (ref. 1). As the result of further work it now seems that the prothrombinase activity is displayed by a complex of activated factor X ($f.X_a$), calcium ions (II) and factor V ($f.V$) which are adsorbed together on a phospholipid surface.

The evidence for this concept is of two kinds. (*a*) It can be shown that $f.X_a$ and $f.V$ are bound by phospholipid micelles, and that calcium ions favour the binding of $f.X_a$. High concentrations of calcium, however, inhibit the binding of $f.V$ (refs. 2–5). (*b*) The kinetics of the generation of prothrombinase activity in mixtures of $f.X_a$, $f.V$, phospholipid and calcium ions can be explained by assuming that a product of all four reactants is the $f.II$ converting substance. It cannot be explained by a reaction of the cascade type[6].

This communication shows that evidence can also be provided for the existence of a complex consisting of the clotting factors IX (in activated form) and VIII, and phospholipid, similar to that described in section (*a*). The interaction between $f.IX_a$ and $f.VIII$ previously has been considered to be of the cascade type, that is

$$\text{VIII} \xrightarrow{IX_a} \text{VIII}_a$$

The experiments described in Table 1, however, show a strong similarity between the characteristics of adsorption onto phospholipid of $f.X_a$ and $f.IX_a$, on the one hand, and of $f.V$ and $f.VIII$, on the other. Calcium ions favour the adsorption of $f.X_a$ and $f.IX_a$; excess calcium inhibits the adsorption of $f.V$ and $f.VIII$. Control experiments show that it is the phospholipid that adsorbs the clotting factors. The activity that disappeared from the super-

natant could be recovered from the resuspended phospholipid sediment. No activity was recorded from the phospholipid in experiments in which no activity was adsorbed from the sample.

Table 1. ADSORPTION OF COAGULATION FACTORS BY PHOSPHOLIPID

Calcium ion conc. (mmolar)	Percentage of clotting factor adsorbed			
	V	VIII	IX	X
1	60	58	27	38
50	<1	41	45	60
100	<1	3	68	76

The figures give the means of three different experiments. Each coagulation factor determination was carried out eight times. The material used as a source of *f*.V and *f*.VIII was barium sulphate adsorbed bovine oxalated plasma. For *f*.IX$_a$ and *f*.X$_a$ oxalated bovine serum was used as a source. To prevent active thrombin being formed from residual amounts of prothrombin, 5 mcg/ml. hirudin was added. The phospholipid used was a suspension of inosithin prepared by homogenizing the crude material in *tris*-HCl buffer 0·02 molar, *p*H 7·5 containing 0·14 molar NaCl. The reaction mixture consisted of 0·9 ml. plasma or serum, 0·4 ml. $CaCl_2$ solution in *tris*-HCl 20 mmolar *p*H 7·5; 0·4 ml. of a suspension of inosithin 5 mg/ml. Half the mixture was centrifuged for 30 min at 100,000*g* at 4° C; in the supernatant the original concentration of phospholipid was restored. The other half of the mixture was stored at 4° C and served as a control. The concentration of the clotting factors in the centrifuged sample was determined and expressed as a percentage of that in the control. The percentage adsorbed was calculated as the amount not recovered in the supernatant. Factors VIII and IX were estimated according to Veltkamp[9], factors V and X were estimated as described in ref. 10. The concentration of hirudin used did not interfere with the clotting factor determinations.

The role of phospholipid in the interaction of *f*.IX$_a$ and *f*.VIII has so far remained obscure, although it has been proved that this role is a mandatory one[7]. Our experiments strongly suggest that phospholipid interacts with calcium ions, *f*.IX$_a$ and *f*.VIII in a manner similar to the interaction of phospholipid, calcium, *f*.X$_a$ and *f*.V. If this is true, the reaction equation of the interaction would be

$$IX_a + VIII + \text{phospholipid} + Ca^{++} \longrightarrow IX_a - Ca - \text{phospholipid} - VIII \text{ (complex } A)$$

Theoretically, it is possible to obtain more evidence for the existence of complex *A* by investigating the kinetics of the generation of *f*.X converting activity from the purified factors IX$_a$ and VIII, that is, by a procedure analogous to the experiments cited in section (*b*). Technical difficulties in obtaining the pure factors IX$_a$ and VIII as well as in the measuring of the conversion *f*.X $\longrightarrow$ *f*.X$_a$ seem to prevent this approach in the near future.

The fact that kinetic evidence obtained with impure systems has so far suggested that *f*.VIII is converted enzymatically by *f*.IX$_a$ (ref. 8) remains disturbing. From the results of experiments on the kinetics of the interaction of factors X$_a$ and V, it was evident that if only a limited range of concentrations was investigated, the results obtained simulated that of an enzyme inter-

action. If the experiments were carried over a wide range of concentrations then the stoichiometric interaction is suggested[6].

We have paid no attention to the form in which *f*.V and *f*.VIII participate in the reactions. By this we do not mean to say that we suppose them to be in the unmodified form in which they occur in intact plasma.

The considerations presented here suggest the following scheme of the intrinsic reaction mechanism of blood coagulation

$$XII \xrightarrow{\text{contact}} XII_a;\ XI \xrightarrow{XII_a} XI_a;\ IX \xrightarrow{XI_a} IX_a$$

$$IX_a + VIII + \text{phospholipid} + Ca^{++} \longrightarrow \text{complex } A$$

$$X \xrightarrow{\text{complex } A} X_a$$

$$X_a + V + \text{phospholipid} + Ca^{++} \longrightarrow \text{complex } B$$

$$II \xrightarrow{\text{complex } B} \text{thrombin}$$

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